

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|---|---|
| (51) International Patent Classification ⁵ : A61K 35/20 | A1 | (11) International Publication Number: WO 95/00155 (43) International Publication Date: 5 January 1995 (05.01.95) |
| (21) International Application Number: PCT/FI94/00252 (22) International Filing Date: 13 June 1994 (13.06.94) (30) Priority Data: 08/080,218 23 June 1993 (23.06.93) US (71) Applicant (for all designated States except US): VALIO BIOTUOTTEET OY [FI/FI]; Tykistökatu 6, Biocity, FIN-20520 Turku (FI). (72) Inventors; and (75) Inventors/Applicants (for US only): LAATO, Matti, Kalevi [FI/FI]; Vahdontie 1, FIN-21270 Nousiainen (FI). JALKANEN, Markku, Tapani [FI/FI]; Rauvolantie 112, FIN-20760 Piispanristi (FI). JALONEN, Harry, Gösta [FI/FI]; Hurtinkatu 11 A 4, FIN-20610 Turku (FI). AALTO, Jouni, Uolevi [FI/FI]; Inkoistenkatu 26 As 2, FIN-21260 Raisio (FI). KANTTINEN, Ari, Perttu, Tapani [FI/FI]; Hämeenkatu 16 C 63, FIN-20500 Turku (FI). PAKKANEN, Raimo, Antero [FI/FI]; Kastovuorenkatu 8 As 2, FIN-21110 Naantali (FI). (74) Agent: TURUN PATENTTITOIMISTO OY; P.O. Box 99, FIN-20521 Turku (FI). | (81) Designated States: AM, AT, AU, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LU, LV, MD, NL, NO, NZ, PL, PT, RO, RU, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report. | |
| (54) Title: METHOD FOR THE IMPROVEMENT OF WOUND HEALING AND COMPOSITIONS THEREFOR | | |
| (57) Abstract <p>The invention relates to a method for the improvement of the healing of wounds in mammals including humans which comprises locally administering to said mammal a safe and effective amount of a colostrum fraction. The colostrum fraction has been prepared by subjecting colostrum, from which part of the fat and cellular debris have been removed by conventional methods, to ultrafiltration by using a membrane having a cut-off of 100,00 Da and recovering the filtrate. The invention includes also pharmaceutical and cosmetic preparations of said colostrum fraction.</p> | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|--|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

METHOD FOR THE IMPROVEMENT OF WOUND HEALING AND
COMPOSITIONS THEREFOR

FIELD OF INVENTION

This invention relates to a method for the improvement of the healing of wounds in mammals including humans by using a colostrum fraction. The invention relates further to
5 pharmaceutical or cosmetic compositions of said colostrum fraction.

BACKGROUND OF THE INVENTION

The term "improving the healing of wounds" in the present text refers to the taking of steps by means of which wounds
10 showing no tendency to heal are induced to start the healing process, and wounds that have started to heal are induced to heal more quickly; or to the taking of steps which are conducive to a cosmetic improvement of the functional result during the healing or after the
15 completion thereof. Improvement of wound healing is particularly important when natural healing is slow or is rendered difficult by a number of negative factors like e.g. infection of the wound, impeded blood flow, medical treatments with cell poisons or with steroids of various
20 kinds, or in cases where patients suffer from chronical disorders with concomitant impairment of normal wound healing e.g. when they are bedridden for prolonged periods of time or suffering from burns, hypertrophic scars, keloids, old age, cancer, diseases giving rise to serious
25 nutritional deficiencies of such types as e.g. chronical inflammatory conditions of the intestine, conditions caused by extensive bodily injuries (so-called polytrauma patients), and comparable type of conditions.

Factors stimulating the healing of wounds have been

described earlier. As examples can be mentioned the epidermal growth factor (EGF), platelet derived growth factor (PDGF) (Grotendorst, J Clin Invest 76:2323, 1985). These factors are known to produce an increased cell
5 division in organ cultures. EGF was thought at the outset to be a promising healing stimulant when tested in experimental studies of its effects in vivo.

The main cell types of skin consists of epidermal keratinocytes and dermal fibroblasts. For wound healing it
10 would be beneficial if the growth and/or migration of epidermal keratinocytes could be promoted. Also for the general skin care keratinocyte growth stimulation can help to maintain the healthy look of skin.

Many known growth factors and other agents known to
15 stimulate the epidermal growth suffer from the disadvantage that they stimulate the growth of the connective (granulation) tissue too strongly compared to their stimulating influence on epidermal (keratin) layer. Excessive collagen synthesis could lead to hypertrophic
20 scars or keloids, such as e.g. in burns. An ideal result would be obtained if the keratin layer grows rapidly while the granulation tissue is growing very slowly or not growing at all. An optimal stimulating agent should therefore exhibit a high growth promoting effect on the
25 keratin layer and a growth inhibiting effect on the granulation tissue.

SUMMARY OF THE INVENTION

The object of the invention is to provide a method for the improvement of the healing of wounds in mammals including
30 humans. As regards the functional result, the main object is to obtain an enhanced growth of epithelial layer while inhibiting the overgrowth of connective tissue.

Another object of the invention is to provide

pharmaceutical or cosmetic compositions, particularly compositions to be used in said method for improving the healing of wounds in mammals including humans.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figures 1A and 1B demonstrate transdermal preparations.

Figure 2 demonstrates the stimulation of keratinocyte DNA synthesis by different concentrations of colostrum fraction.

Figure 3 discloses proliferation of HaCat cells in vitro in
10 10 % FCS (triangles), 1 % adult bovine serum (circles) and
1 % adult bovine serum + 5 % CF (squares).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The inventors have found that a colostrum fraction containing the growth factors of colostrum stimulate the
15 growth of the keratin layer very strongly while it
simultaneously inhibits the growth of the granulation
tissue.

Bovine colostrum fractions rich in growth factors are known as excellent additives to cell culture media to promote the
20 growth of cells, particularly hybridoma cells. Reference is made to R Pakkanen et al, Appl Microbiol Biotechnol (1992) 37: 451-456 and International Patent Application No PCT/FI92/00268. These publications describe the preparation of the colostrum fraction which has very low concentrations
25 of endotoxin, protein and immunoglobulin but which contains growth factors, trace elements, vitamins and other small-molecular compounds essential for cell growth present in colostrum.

The invention thus concern a method for improving the
30 healing of wounds in mammals including humans which

comprises locally administering to said mammal a safe and effective amount of a colostrum fraction, said colostrum fraction having been prepared by subjecting colostrum, from which part of the fat and cellular debris have been removed by conventional methods such as centrifugation, to
5 ultrafiltration by using a membrane having a cut off of 100,000 Da and recovering the filtrate.

The invention further concerns a pharmaceutical or cosmetic composition, particularly a composition for improving the
10 healing of wounds in mammals including humans comprising a colostrum fraction, said colostrum fraction having been prepared by subjecting colostrum, from which part of the fat and cellular debris have been removed by conventional methods, to ultrafiltration by using a membrane having a
15 cut off of 100,000 Da and recovering the filtrate, and a compatible non-toxic carrier therefor.

According to a preferred embodiment, casein has been removed from the colostrum by precipitation previous to its ultrafiltration. The removal of casein results in lower
20 endotoxin and immunoglobulin contents in the ultrafiltrated colostrum fraction.

The colostrum fraction is for practical reasons preferably obtained from bovine colostrum. Colostrum from other sources is, however, also believed to be useful for the
25 preparation of the colostrum fraction to be used in this invention.

The topical or transdermal administration of the colostrum fraction can be accomplished mainly in three different ways: (i) by mixing (or adsorbing) an effective amount of
30 the colostrum fraction with suitable non-toxic carriers and optionally penetration enhancers to form ointments, emulsions, solutions, suspensions, lotions, creams, gels, powders, aerosols, bandage patches or the like, where preferably a fixed amount of said formulation is to be

applied onto the wound, or (ii) by incorporating the colostrum fraction into a transdermal delivery system according to known technology, or (iii) by incorporating the bioactive substances from the colostrum fraction into liposomes which can either be used as such or can be incorporated in the formulations of the previous alternatives (i) or (ii).

In the compositions of this invention, the role of the transdermal administration is mainly to transport the colostrum fraction through the epidermal layer and not necessarily to achieve a systemic effect.

Examples of suitable excipients include those well known in the art of pharmacy and cosmetology for the preparation of topical formulations such as aqueous buffered saline solutions, non-volatile fatty alcohols, acids, esters, e.g. cetostearyl alcohol and cetyl alcohol; volatile alcoholic compounds, e.g. ethanol and isopropanol; glycols and glycol ethers.

The healing of wounds in the stomach or the intestine, i.e. peptic ulcers can also be improved by use of the method according to the invention. For the treatment of peptic ulcers the colostrum fraction can be used as such combined with flavouring agents and preservatives. Alternatively, the colostrum fraction can be absorbed into particles or into a matrix, which optionally could be coated with a material having slow release or enteric properties. The matrix or particles can further be pressed into tablets or put into capsules.

The following non-limiting examples demonstrate the compositions according to the invention.

Reference example

Whey was prepared from defatted colostrum by removal of

casein using acid precipitation, (see, for example, R. Pakkanen et al., Appl. Microbiol Biotechnol (1992) 37; p. 452.). An ultrafiltrate was obtained from cleared whey by filtration through membranes with a nominal molecular weight cut-off of 100,000 Da. The colostrum ultrafiltrate (colostrum fraction) so obtained contained 1.1g g/l protein, 0.24 g/l immunoglobulin G (IgG) and less than 0.24 EU (endotoxin unit)/ml endotoxins.

Example 1

10 Examples of semisolid preparations

| | | | |
|----|----|-----------------------------------|-------|
| 15 | a) | White petrolatum | 20 g |
| | | Stearyl alcohol | 25 g |
| | | Propylene glycol | 10 g |
| | | Sodium lauryl sulfate | 1 g |
| | | Methyl paraben | 25 mg |
| | | Propyl paraben | 15 mg |
| | | Distilled water | 34 ml |
| | | Colostrum fraction | 10 ml |
| 20 | b) | Polyethylene glycol 4000 | 45 g |
| | | Polyethylene glycol 400 | 45 g |
| | | Colostrum fraction | 10 ml |
| 25 | c) | Methocel 90 H.C. 4000 | 1 g |
| | | Carbopol 934 | 24 mg |
| | | Propylene glycol | 18 g |
| | | Methyl paraben | 15 mg |
| | | Distilled water | 71 ml |
| | | Colostrum fraction | 10 ml |
| | | NaOH added to adjust the pH to 7. | |

Example 2

Transdermal formulations

The colostrum fraction can be incorporated into a transdermal drug delivery device. Such devices are illustrated in Fig. 1A and 1B, where reference number 1 represents an backing layer, 2 is an adhesive layer, which keeps the patch attached to the skin and 3 is a porous polymer. In Fig. 1A the colostrum fraction has been absorbed into the porous polymer 3, either alone or in combination with a vehicle and/or a penetration enhancer. In Fig. 1B the reservoir 4 contains the colostrum fraction solution.

Example 3

Liposome formulations

| | | |
|----|--------------------------|-------|
| 15 | Colostrum fraction | 1 ml |
| | Phosphatidyl choline | 70 mg |
| | Phosphatidyl serine | 20 mg |
| | Cholesterol | 10 mg |
| | Buffered distilled water | 10 ml |

- 20 The best way to prepare liposomes of the above mixture is either the use of the French press or the membrane extrusion method. Alternatively, it is possible to prepare unloaded liposomes according to known methods and later add the bioactive components of the colostrum fraction by using
- 25 pH gradients according to the active loading principles (R C New, Liposomes - a practical approach, IRL Press at Oxford University Press).

The required dose of the colostrum fraction will vary especially with the particular kind of wound to be treated,

30 the severity of its condition and the dosage form. We

believe that a suitable dose could range from some millilitres to one litre or more of colostrum fraction per day and adult person. The preferred dose for topical or transdermal preparations would be about 0.5 - 1 ml/day and 5 cm² of wound area. For the treatment of peptic ulcers the preferable oral dosage is believed to be about 50 to 1000 ml per day and adult person.

The therapeutic effect of the colostrum fraction is demonstrated by the following non-limiting examples. The colostrum fraction used in the experiments had an endotoxin 10 content of 1.0 EU/ml, a total protein content of 2.0 mg/ml and an immunoglobulin content of 0.25 mg/ml. The fraction can was obtained by subjecting the defatted colostrum to casein precipitation before the ultrafiltration.

15 Example 4

Treatment of keratinocyte cultures with colostrum fraction (CF) solutions

The effect of the colostrum fraction (CF) was therefore studied on keratinocyte growth stimulation. This was done 20 with classical cell culture method, where cultured keratinocytes were exposed to various concentration of CF. A human keratinocyte cell line (HaCat: Fusenig and Worst Exp. Cell Res. 93:443-457, 1992) was cultured routinely in modified Eagle's MEM supplemented with 10 % FCS (fetal calf 25 serum) and antibiotics at 37°C, 5 % CO₂ and 95 % air in a humidified incubator as described (Elenius et al. J. Biol. Chem., 265: 17837-17843, 1990). In the first experiment, HaCat cells were divided into petri dishes and grown until semi-confluent. After that cells were maintained 24 h in 30 serum free medium following the addition of different concentrations of CF and FCS. Cells were incubated with these solutions for next 24 h after which ¹²⁵I-deoxiuridine was added for an additional 2 hours. Finally cells were washed with cold PBS, fixed and extracted into 0.2 M NaOH.

Radioactivity remaining in the cell extracts was measured with a gamma counter.

Figure 2 demonstrates the stimulation of keratinocyte DNA synthesis by CF. Human keratinocytes were plated on culture dishes and grown to semiconfluency in the presence of 10 % FCS. After 24-h starvation in plain culture medium, media containing different concentrations of CF or 10 % FCS were added for following 24 hours. At the end of the incubation DNA synthesis rates were measured by adding ^{125}I -deoxyuridine into cultures for additional two hours and measuring the rates of IdU incorporations. Mean \pm S.D. of four samples are presented. C = plain medium; 1 % M = 1 % CF; 5 % M = 5 % CF; 10 % M = 10 % CF; 10 % FCS = 10 % fetal calf serum.

As shown in Figure 2, this analysis revealed a stimulation of DNA synthesis in the presence of increasing concentrations of CF. This stimulation was almost as high as stimulation obtained with fetal calf serum, the best growth supported for cultured cells. These results clearly indicate that CF contains factors which can stimulate DNA synthesis of keratinocytes.

In the second experiment HaCat cells were suspended in modified Eagle's MEM supplemented with 1 % adult bovine serum and antibiotics and divided into 24-well cell culture plates at a concentration of 30 000 viable cells/ml (1.5 ml/well). The cells were incubated at 37°C, 5 % CO₂ and 95 % air for 24 hours. Then, the media were removed from the wells and 1.5 ml samples of test media were added (10 % FCS, 1 % adult bovine serum, 5 % CF + 1 % adult bovine serum in modified Eagle's MEM supplemented with antibiotics). At the time points indicated (Fig. 3) the attached cells were detached with trypsin and counted. The old media samples of the other wells were also replaced with fresh samples at the same days. Cell counts were performed in a haemocytometer using trypan blue exclusion

to determine viability and each cell was counted only once. The experiment was performed in duplicate.

As shown in Fig. 3 the results indicate that CF contains growth factors which stimulate proliferation of HaCat cells in vitro. Moreover, the results suggest that these factors are either not present in normal adult bovine serum or their concentration is significantly higher in CF.

Example 5

Effect of the colostrum fraction on the granulation tissue.

10 The effect of the colostrum fraction (CF) as described in the foregoing example on the wound healing improvement was studied in a standardized experimental wound model in rats as described by Niinikoski, Heughan and Hunt (Surg Gynecol Obstet 133: 1003-1007, 1971). This model could briefly be
15 described as follows:

Treatment of the wound with solutions of a colostrum fraction (CF):

Viscose cellulose sponge (Säteri Oy, Valkeakoski, Finland) was used as an inductive matrix for repair tissue. The
20 material was cut into cylindrical pieces, 40 mm long and 10 mm in diameter, and a tunnel of 3 mm in diameter was made through the center of the sponge. Silicone rubber discs, 10 mm in diameter and 2 mm thick, were stitched onto both ends of the sponge to create a stable dead space. The cylinders
25 were decontaminated by boiling for 30 min in physiological saline and the implantations were performed with strictly aseptic techniques. Male Sprague Dawley rats weighing 230 - 250 g were anesthetized with ether and an incision, 4 cm long, was made in the dorsal midline at the caudal portion
30 of the back. Each rat received one sponge cylinder that was implanted longitudinally under the skin, cephalad from the incision. During the experiments the animals received a

normal rat diet and water ad libitum and were housed individually in cages in the animal quarters. The design of the work was approved by the local ethical committee.

In vivo experiments:

- 5 Altogether 32 rats were studied in four groups of 8 animals. In the three test groups the implants were injected immediately after implantation with 1 ml of 1, 5 or 10% of colostrum fraction (CF) in phosphate buffer saline (PBS) into the central tunnel. The control group was
- 10 injected similarly with the carrier solution only. Injections of all the groups were repeated daily under strictly aseptic conditions. No infections were observed. After collection of the wound fluid samples the rats were anesthetized with ether and sacrificed. The implants were
- 15 dissected free from the surrounding tissue, and the silicone rubber discs were removed. Nucleic acids were extracted from the implants according to the method of Schmidt and Thannhauser. DNA was determined by the diphenylamine reaction and RNA was assayed as RNA-ribose by
- 20 the method of Ceriotti. Aliquots were taken for the determination of nitrogen, hydroxyproline, hexosamines and uronic acids (Laato M, Acta Chir Scand Suppl 546: 1-44, 1988).

The results are given in Table 1. The units are mg/sponge

25 and the abbreviations have the following meaning: CF = colostrum fraction; HYPRO = hydroxypropylene; HEXOS = hexosamines and URONIC = uronic acid.

Table 1

| | DNA | RNA-Ribose | NITROGEN | HYPRO | HEXOS | URONIC |
|---------|---------|------------|----------|---------|---------|---------|
| Groups | Average | Average | Average | Average | Average | Average |
| control | 33,825 | 5,5625 | 82,6 | 10,005 | 6,875 | 6,825 |
| 1% CF | 36,125 | 6,0375 | 82,5625 | 9,725 | 6,575 | 7,2625 |
| 5% CF | 28,275 | 6,5375 | 89,9125 | 7,13 | 5,2875 | 6,2375 |
| 10% CF | 25,8875 | 6,5875 | 83,425 | 8,25 | 6,375 | 6,8 |
| | | | | | | |
| | P-value | P-value | P-value | P-value | P-value | P-value |
| | 0,01285 | 0,00515 | 0,18347 | 0,24312 | 0,03023 | 0,07885 |

CLAIMS

1. A method for the improvement of the healing of wounds in mammals including humans which comprises locally administering to said mammal a safe and effective amount of
5. a colostrum fraction, said colostrum fraction having been prepared by subjecting colostrum, from which part of the fat and cellular debris have been removed by conventional methods, to ultrafiltration by using a membrane having a cut off of 100,000 Da and recovering the filtrate.
- 10 2. The method according to claim 1 wherein casein has been removed from the colostrum by precipitation previous ultrafiltration.
3. The method according to claim 2 wherein the colostrum fraction is a bovine colostrum fraction.
- 15 4. The method according to claim 3 wherein the wound is a topical wound.
5. The method according to claim 3 wherein the wound is a peptic ulcer.
- 20 6. A pharmaceutical or cosmetic composition comprising a colostrum fraction, said colostrum fraction having been prepared by subjecting colostrum, from which part of the fat and cellular debris have been removed by conventional methods, to ultrafiltration by using a membrane having a cut off of 100,000 Da and recovering the filtrate, and a
- 25 compatible non-toxic carrier therefor.
7. A composition for the improvement of the healing of wounds in mammals including humans comprising a colostrum fraction, said colostrum fraction having been prepared by subjecting colostrum, from which part of the fat and
- 30 cellular debris have been removed by conventional methods, to ultrafiltration by using a membrane having a cut off of

100,000 Da and recovering the filtrate, and a compatible non-toxic carrier therefor.

8. The composition according to claim 7 wherein casein has been removed from the colostrum by precipitation previous
5 ultrafiltration.

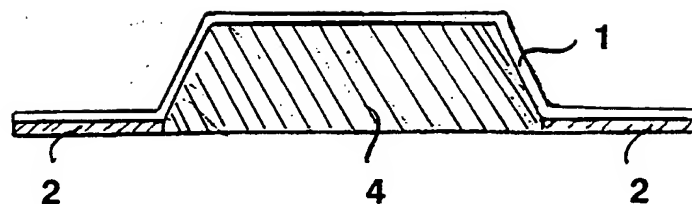
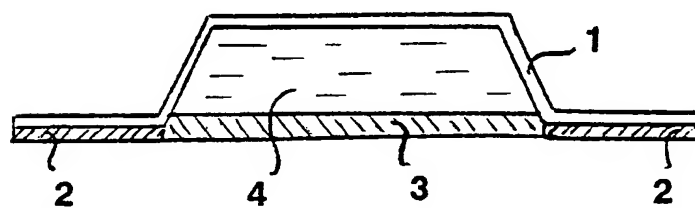
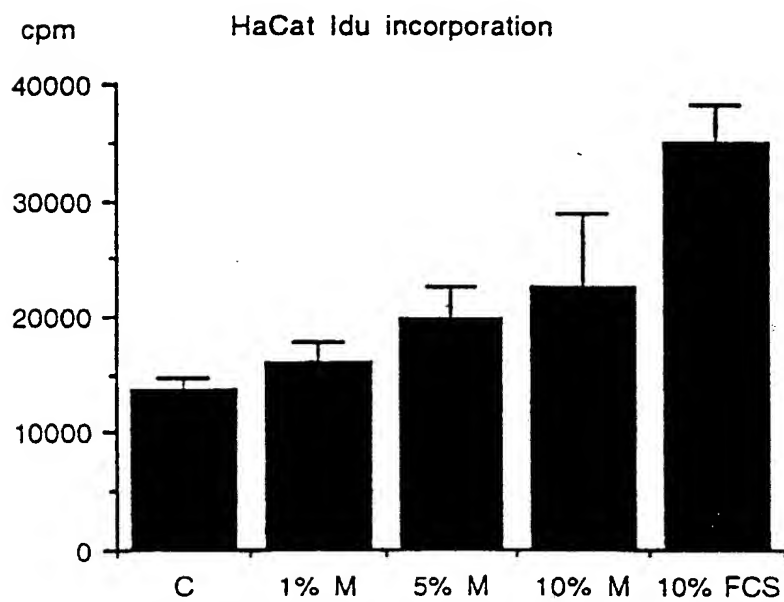
9. The composition according to claim 8 wherein the colostrum fraction is a bovine colostrum fraction.

10. The composition according to claim 7 which is in the form of ointment, emulsion, suspension, lotion, solution,
10 gel, cream, powder or aerosol.

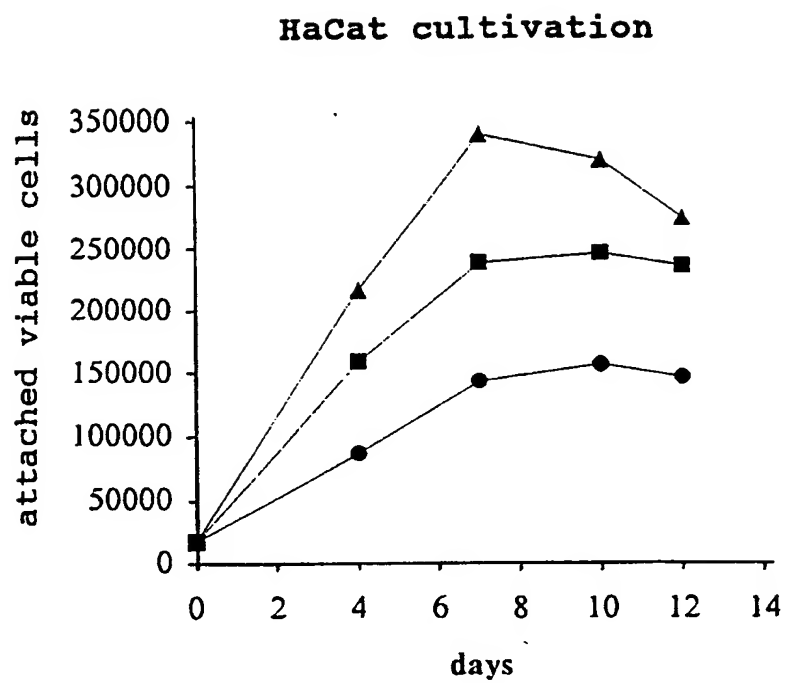
11. The composition according to claim 7 which is a transdermal delivery system.

12. The composition according to claims 7 which is in the form of a tablet or capsule for oral administration.

1/2

**Fig. 1A****Fig. 1B****Fig. 2**

2/2

**Fig. 3**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 94/00252

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 35/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| X | EP, A2, 0334776 (GATTEFOSSE S.A.), 27 Sept 1989 (27.09.89) -- | 6-11 |
| X | FR, A1, 2460135 (LIOTET SERGE ET AL), 23 January 1981 (23.01.81) -- | 6-11 |
| X | US, A, 4342747 (SERGE LIOTET ET AL), 3 August 1982 (03.08.82) -- | 6-11 |
| X | Derwent's abstract, No 92-248203/30, week 9230, ABSTRACT OF SU, 1685253 (KOZLOV V K), 15 October 1991 (15.10.91) -- | 6-11 |



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 Sept. 1994

Date of mailing of the international search report

06-10-1994

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Göran Karlsson
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 94/00252

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|--|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | FR, A1, 2627386 (LIOTET SERGE ET AL), 25 August 1989 (25.08.89) -- | 6-11 |
| A | FI, A, 893404 (VALIO MEIJERIEN KESKUSOSUUSLIIKE), 14 January 1991 (14.01.91) -- ----- | 6-10,12 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 94/00252

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-5
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy,
see rule 39.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.